1975

The Histochemical Characterization of Rat Masseter Muscle Fiber Types

Robert George Thomas
Loyola University Chicago

THE HISTOCHEMICAL CHARACTERIZATION OF RAT MASSETER MUSCLE FIBER TYPES

by

Robert G. Thomas

A Thesis Submitted to the Faculty of the Graduate School of Loyola University in Partial Fulfillment of the Requirements for the Degree of Master of Science

June

1975

ACKNOWLEDGEMENTS

I wish to extend my sincere gratitude to my advisors, Dr. Patrick Toto and Dr. William F. Malone for their interest and guidance in this thesis.

I also wish to express my appreciation to Mrs. Valeria Lescinskas for her technical assistance and Helen Zaidan for her help in preparing this manuscript.

AUTOBIOGRAPHY

Robert George Thomas, the son of Mr. and Mrs. Robert Thomas was born in Oak Park, Illinois on November 5, 1945. He has a younger brother, Paul, who graduated from the University of Illinois as a computer engineer in 1974.

After graduation from Hoopeston Senior High School, Hoopeston, Ill., he received the Frank Gannett Scholarship and the Illinois General Assembly Scholarship. In 1968 he graduated from the University of Illinois with a Bachelor of Science degree. In 1970 he received his dental degree from the University of Illinois Dental School.

He and his wife, Connie, have two children, Robert and Hilary.

After graduation Dr. Thomas became an intern at the Veteran's Administration Research Hospital, Chicago, Illinois. Upon completion of his internship be began his studies in Fixed Prosthodontics and Oral Biology at Loyola University. In 1972 he started his residency in Orthodontics at Loyola. He completed his training in June, 1974.

At present he is Assistant Professor and Assistant Chairman of the Department of Orthodontics at Loyola University.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

I. INTRODUCTORY REMARKS AND STATEMENT OF THE PROBLEM

The specific intent of this study was to determine the existence of intermediate fibers in the masseter muscle of the Sprague-Dawley rat. The intermediate fibers of the muscles of mastication have never been unequivocally characterized into a distinct group. Muscle imbalance has been empirically cited as the primary etiology for temporomandibular joint disturbance and orthodontic relapse. It is impossible to assess the extent of muscle involvement in treatment problems until more information about muscle is available. The characterization of the muscles of mastication into distinct fiber types and their ultimate correlation with muscle physiology will permit a more objective assessment of the involvement of muscle in dental therapy. This study will involve a histochemical characterization of rat masseter muscle fiber types utilizing techniques to detect the presence of succinic dehydrogenase, phosphorylase and adenosine triphosphatase. The method for adenosine triphosphatase is essential to verify the presence of the intermediate muscle fiber type in the muscles of mastication which to date has not been reported in the literature.

II. REVIEW OF THE LITERATURE

Mammalian muscles differ in color from near white to deep red. Also in a given animal species some skeletal muscles contract more quickly than others. Kuhne (1865) showed that color difference was a property of the muscle fibers, not their blood content. Ranvier (1873) showed that certain red muscles contracted much more slowly than white muscles. This has been repeatedly confirmed by Kronecker and Stirling (1878), Fischer (1908), Denny-Brown (1929) and Cooper and Eccles (1930). However, the color of a muscle could not always be associated with a particular speed or fiber structure (Meyer 1875).

Paukul (1904), using the rabbit, clearly showed that slow-twitch muscles such as the soleus and ischio-tibialis are always red, but that not all red muscles are slow. He found that the time course of the twitch of the red masseter muscle is about the same as that of fast-twitch muscles such as the medial gastrocnemius. Lankester (1871) and Knoll (1891) observed that red pigment is present in those muscles involved in continuous activity.

Grutzer and others as early as 1884 revealed that mammalian skeletal muscles are usually heterogeneous. That is they contain two kinds of fibers: the red fibers and the white fibers. The red fibers were usually thin and dark and contained many mitochondria and fat droplets; the white fibers were thick, appeared clear and contained few mitochondria and fat droplets.

The only other study of the muscles of mastication was by Baker and Laskin (1968). However, the presence of the intermediate fiber type was alluded to but was not specifically shown in their research. Two enzyme tests were used to classify the fiber types present (red or white) according

to a percentage ratio of red to white fibers. The intermediate fiber types were not described as a definite entity. Stein and Padykula (1962), Romanul (1964), Ogata (1964), Edstrom and Kugelberg (1968), Engel (1968), Edstrom and Nystrom (1969), Edgerton (1969), Eversole (1970), Yellin (1970), Barnard et. al. (1971), Close (1972) and others have shown the presence of the intermediate fiber.

Edgerton and Simpson (1969) have shown on the basis of oxidative enzyme staining, that a two-category classification of fibers as simple red or white does not adequately describe their metabolic observations.

Both the common practice to consider intermediate fibers as red fibers when using a two-category scale (James, 1968, Stein and Padykula, 1962) and the erroneous assumption that intermediate fibers are intermediate in contractile properties while red fibers are slowest (Jinnai, 1960) have led to more confusion.

It has been unquestionably shown that muscles consisting almost wholly of red fibers can be as fast or faster than some representative white muscles. The thyroarytenoid of the rabbit, an extremely fast muscle is histochemically a red muscle (Hall-Craggs, 1968). However, the rat soleus muscle which consists primarily of intermediate fibers and a few red fibers contracts very slowly in comparison to the gastrocnemius, a white muscle (Close, 1967). Also the research of Stein and Padykula (1962) indicated to Close that the intermediate fiber motor units were slower than the red fiber motor units.

Many nomenclatures of the three fiber types have been described in various studies (white, medium, red (Ogata, 1958; Ogata and Mori, 1964); A, B and C (Stein and Padykula, 1962); I, III, II (Romanul, 1964); II, I, II (Engel, 1962) fast-twitch white, slow-twitch intermediate and fast-twitch red

(Barnard et. al., 1971) include the major classifications. For this study the terms white, intermediate and red will be used throughout.

In general it has been stated by Close (1972) that white fibers have a high glycolytic, low oxidative and high myofibrillar ATPase activities, that intermediate fibers have low glycolytic, intermediate oxidative and low myofibrillar ATPase activities and that red fibers have intermediate glycolytic, high oxidative, and high myofibrillar ATPase activities.

Intermediate fibers may be distinguished from white and red fibers on the basis of intensity of stain for myosin ATPase and white fibers show a much lighter staining reaction for oxidative enzymes than either intermediate or red fibers.

Eversole and Standish (1970) have shown the presence of the intermediate fiber in semimembranosis M by using ATPase, phosphorylase and succinic dehydrogenase tests. They first classified the three fiber types according to their stain intensity for succinic dehydrogenase. The red fibers were small and densely stained, followed by the intermediate fibers with a moderate reaction and size. The white fibers showed very little staining intensity and were large in diameter.

Stein and Padykula (1962) classified the three muscle fiber types according to the distribution of diformazan granules.

White fibers are characterized by:

1. Lack of subsarcolemmal activity
2. Particles arranged in an open network
3. Particles arranged in streaks which are sometimes parallel and at other times meet at junctions marked by a larger particle creating a stellate configuration.

Intermediate fiber types are characterized by:

1. Lack of strong subsarcolemmal activity
2. Particles arranged mostly in the form of small polygons.

Red fibers are characterized by:

1. Very strong subsarcolemmal SDH activity with elongated masses of diformazan.
2. Large spherical particles preferentially arranged toward the fiber periphery.
3. Particles aligned in short palisaded streaks.

III. MATERIALS AND METHODS

A. Experimental Animals

Six young adult Spague-Dawley rats were utilized. Both masseter muscles of each rat were used.

B. Preparation of Tissue

The muscle specimens were obtained early in the morning to reduce the effect of recent mastication on enzyme activity (Gollick, 1961; Gould, 1959; Hearn, 1957; Kendrick-Jones, 1965; Romanul, 1964; Baker, 1968).

Each rat was sacrificed utilizing ether in a dessicator. The animals were surgerized immediately. Their right and left masseter muscles were removed in their entirety.

The rat masseter is divisible into three main parts, the pars superficialis, pars xygomaticus and pars major (Howell, 1926). Sections were taken from the area where all three divisions come together to obtain a representation of the entire muscle (see Figure I). The specimens were cut with sharp dissection as perpendicular to the fiber direction as possible.

The specimens were placed on cryostat buttons and immediately frozen to -60^{0}C for sectioning. It has been shown repeatedly that quick frozen, unfixed tissue will provide excellent recovery of all three enzymes being studied (Barka, 1963; Beatty, 1966; Chang, 1961; Diculesco, 1964; Dobowitz, 1960; Feder, 1958; Greiff, 1966; Hori, 1964; Horowitz, 1967; Melnick, 1965; Meryman, 1960; Nachlas, 1956; Novikoff, 1960; Ohkawa, 1965; Takeuchi, 1955; Wachstein, 1955).

Four serial 6 micron sections from each specimen were placed on six separate slides. The slides were then incubated for succinic dehydrogenase,

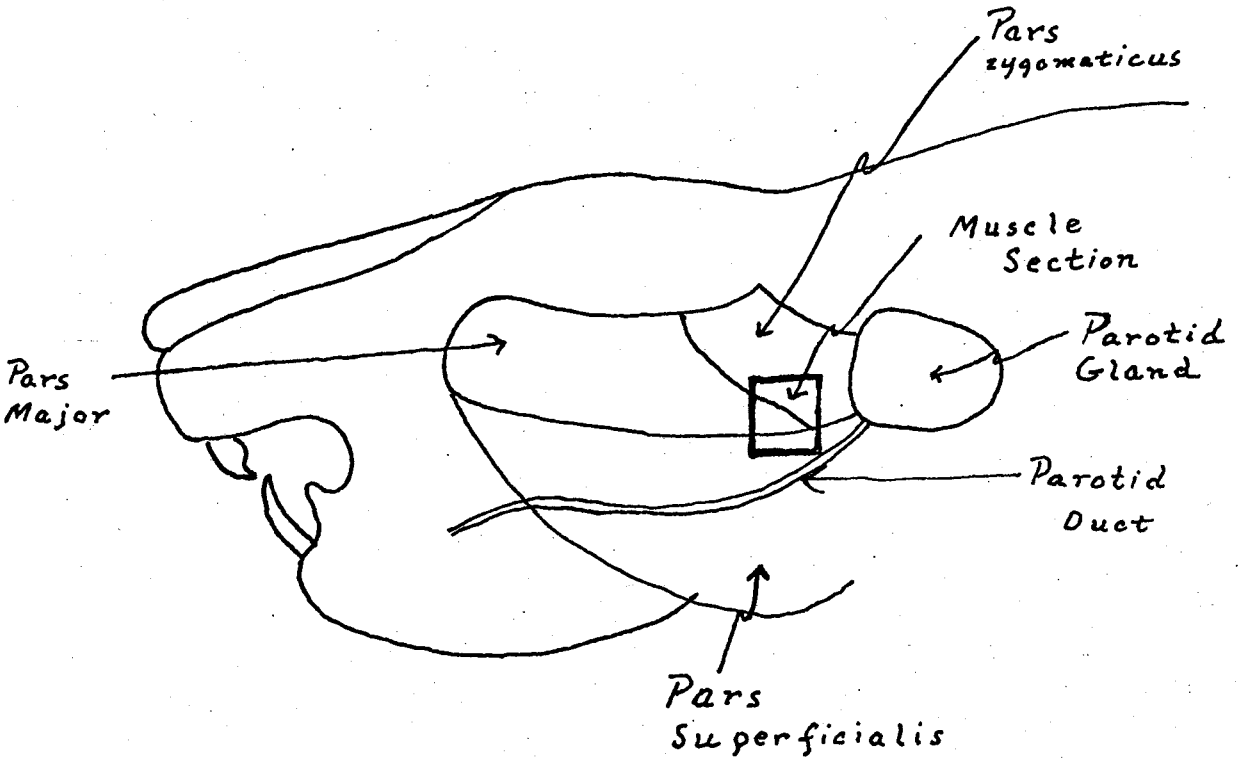

Fig. I. Diagram of Sprague-Dawley rat showing three divisions of rat masseter muscle.

phosphorylase and myosin ATPase.

Two critical pilot studies were carried out to establish ideal incubation periods and reproducibility of the results.

C. Incubation Techniques

1. Succinic dehydrogenase

For demonstration of succinic dehydrogenase activity the method of Nachlas (1956) utilizing a p-nitrophenyl ditetrazole, 2,2'-di-p-nitrophenyl-5-5'-diphenyl-3,3'dimethoxy-4,4l-biphenylene) ditetrazolium chloride (Nitro-BT) was utilized.

The detection of succinic dehydrogenase activity was based on the knowledge that this enzyme catalyses the reversible oxidation of succinate to fumarate by effecting a transfer of hydrogen ions from succinate to the cytochrome system (Barka, 1963; Kleiner, 1962). Its histochemical localization depends on the reduction of a water soluble, colorless tetrazolium salt, nitroblue tetrazolium, to a water insoluble blue colored formazan dye (Malaty, 1953; Baker, 1968).

A stock solution of buffered succinate was prepared in advance by combining equal volumes of phosphate buffer (0.2M) at pH7.6 and sodium succinate (0.2M). Before sectioning the incubation media was formulated by adding 10 ml. of the buffered substrate reagent to 10 ml. of an aqueous solution containing 10 mg. of Nitro-BT.

The fresh cut and thawed sections were incubated for 30 minutes at 37°C. They were washed in saline, fixed in 10% formol-saline for 10 minutes, washed in 15% alcohol for 10 minutes, rinsed with distilled water and mounted in glycerine jelly. Control sections were prepared exactly the same with the exception of Nitro-BT which was excluded.

They were immediately read and photographed to prevent any changes on the slides.

2. Phosphorylase

To study phosphorylase activity the method of Eranko and Palkama (1961) modified after Takeuchi and Kuriaki (1955) was used.

In vivo, phosphorylase (non-branching or amylophosphorylase) catalyses the synthesis of alpha 1-4 glucosidic bonds at the non-reducing ends of the polysaccharide substrate producing a linear chain molecule (Takeuchi, 1958). Once a critical length is acheived, the branching phosphorylase enzyme (amylo, 1-4, 1-6 transglucosidase or Q-enzyme) acts to produce branching glycogen linkages by joining alpha 1-6 glucosidic bonds (Stubbs, 1965, Baker, 1968).

A mixture of 100 mg. glucose-1-phosphate, 10 mg. adenosine-5-phosphate, 2 mg. glycogen, 180 mg. sodium fluoride, 900 mg. polyvinyl pyrrolidone, 1 drop of 40 1.u./ml. insulin, 2 ml. absolute alcohol, and 10 ml. 0.1M acetate buffer at pH 5.9 was freshly made and filtered. Then freshly made sections 6 microns thick were incubated for 30 minutes at 37°C. The slides were allowed to dry and immersed in 40% alcohol for 2 minutes. They were again allowed to dry and immersed in 0.32M sucrose for 5 minutes. They were then stained with Gram's iodine solution, made in 0.32M sucrose for 5 minutes and mounted in iodine-glycerol. Control sections were treated identically, but glucose-1-phosphate was eliminated. Polyvinyl pyrrolidone was used to increase the viscosity of the incubation media and, therefore, reduce the amount of diffusion of the reaction products.

For control slides glucose-1-phosphate was eliminated from the incubation solution.

3. ATPase

The first attempt at studying adenosine triphosphatase histochemically was by Glick and Fischer (1945). The method was abandoned because the use of paraffin embedding has been shown to inhibit enzyme activity. In 1952 Maengwyn-Davis, Friedenwald, and White performed this staining reaction on unfixed frozen sections avoiding any inactivation which might have resulted from fixation or paraffin embedding. However, lengthy incubation periods were necessary. The reported instability of ATP in solution at 37°C (Morell et. al., 1951, Wade and Morgan, 1954) and the complications of long incubation times (Herman and Deane, 1953) led Padykula and Herman (1955) to improve the sensitivity of activity of ATPase.

Therefore, the method of Padykula and Herman was used. Twenty ml. of 0.1M sodium barbital and 10 ml. of 0.18M calcium chloride was added to 30ml. of distilled water. ATP in dry form was added until the final concentration was 0.012M. The pH was adjusted to 9.4 with 0.1M sodium hydroxide. The slides were then incubated for 30 minutes.

They were then washed with three changes of one percent aqueous calcium chloride for ten minutes. They were transferred to 2% cobaltous chloride for three minutes and washed with several changes of distilled water. The slides were immersed in 1% yellow ammonium sulfide for two minutes, washed well, dehydrated with 80%, 95% and 100% ethanol and cleared with xylol.

For control sections, adenosine triphosphate was left out of the incubation medium.

IV. RESULTS

The method of Eversole and Standish was used to identify the three fiber types. The fibers were first identified by differences in intensity of stain correlated with the appropriate fiber size. It has also been shown that red and white fibers display a reciprocal relationship in comparison with phosphorylase localizations. A negative reaction for ATPase indicates that the fiber is definitely an intermediate one (see Table I). This was clearly shown with both ATPase and SDH.

A. Succinic Dehydrogenase

As is shown in Figure II intermediate, white and red fiber types were observed. This was true of all sections stained.

The intermediate fibers were intermediate in stain density and size. The enzyme activity was moderate and was evenly dispersed throughout the fiber. Fine diformazan granules were present.

The red fiber types were very densely stained and showed course diformazan granules. They were very easy to recognize because of their small diameter. Their diformazan granules were mainly subsarcolemmal.

The white fiber types showed minimal succinic dehydrogenase activity. They were large in diameter and there was little subsarcolemmal activity present.

All control sections were clear and there was no evidence of reaction.

B. Myosin ATPase

As stated by Eversole and Standish a negative reaction for ATPase clearly indicated the presence of intermediate fibers. Their presence

TABLE I

Characteristics of Rat Masseter Muscle Fiber Types.

	RED FIBERS	INTERMEDIATE FIBERS	WHITE FIBERS
SDH	Positive	Moderate Reaction	Negative
ATP	Positive	Variable, negative is always an intermediate fiber	Positive
Phosphorylase	Negative Always	Variable (negative or positive)	Large
Contraction Time	Varies, usually slow but can be fast	Slow	Fast usually but can be slow

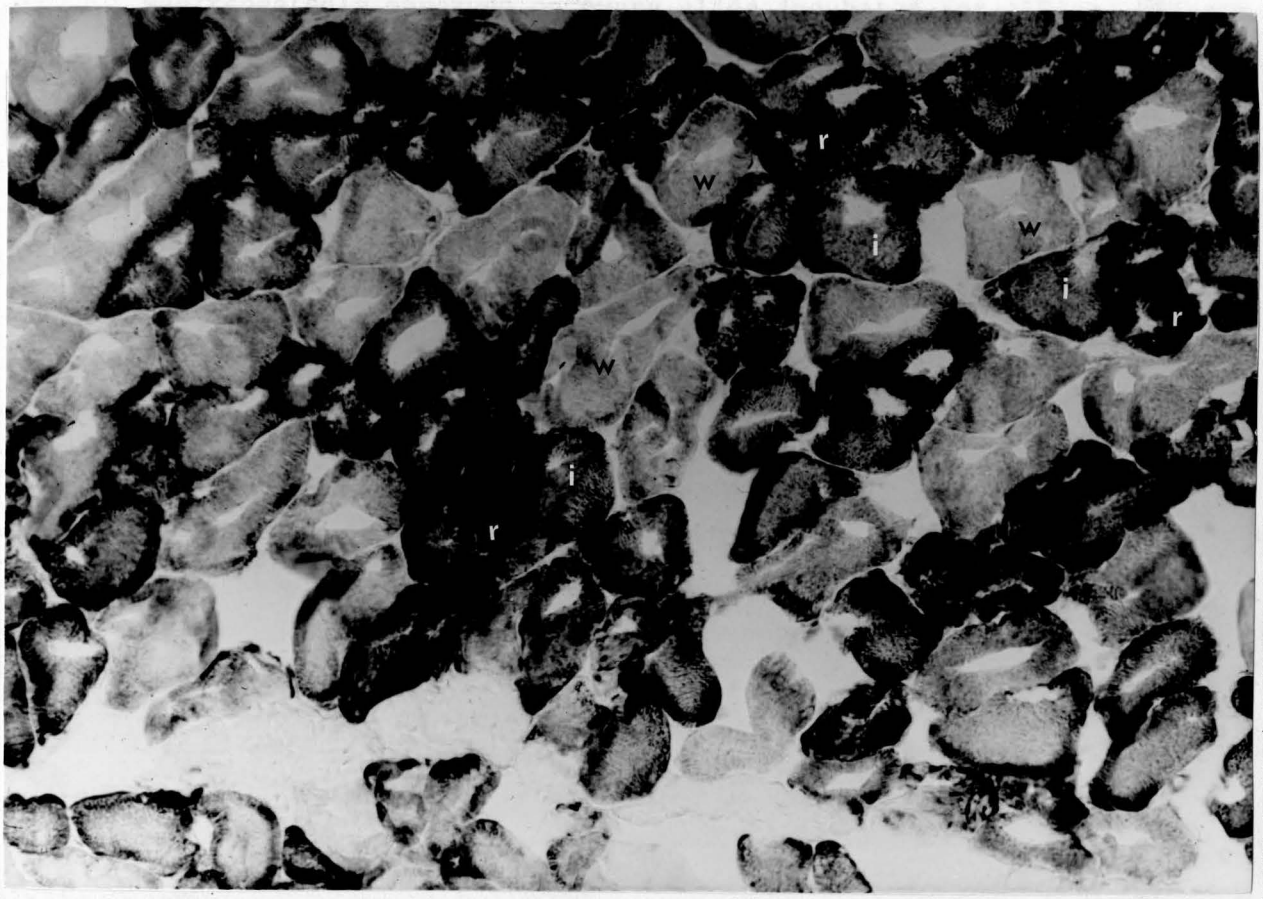

Fig. II. Incubation with Succinic Dehydrogenase. Intermediate fibers are labeled i; red fibers are labeled r; white fibers are labeled w. Note the density of stain; size of fiber and concentration areas of diformazan granules.

was repeatedly observed on every slide incubated for ATPase. Figures III and IV show the type of negative reaction observed. It should also be pointed out that some intermediate fibers are variable to staining density with ATPase. That is some of the fibers present may still be intermediate fibers even though they are not negative for ATPase. Also, both red and white fibers stain positively for ATPase.

C. Phosphorylase

Figures V and VI clearly show that white fibers are present in rat masseter muscle. They were densely stained after 30 minutes of incubation time. Red fibers showed a negative reaction. However, intermediate fibers were variable. They reacted both negatively and positively. As a general rule it was observed that intermediate fibers which were negative for ATPase were also positive for phosphorylase in many instances. Further study is required to confirm this observation.

Note that the smaller fibers observed in Figure VI are negative. This was another indication that they were definitely red fibers.

Fig. III. Incubation with Adenosine Triphosphatase. The negative fibers are definitely intermediate fibers. The positive fibers may be red, white or intermediate fibers.

Fig. IV. Incubation with Adenosine Triphosphatase. Note the intermediate fibers.

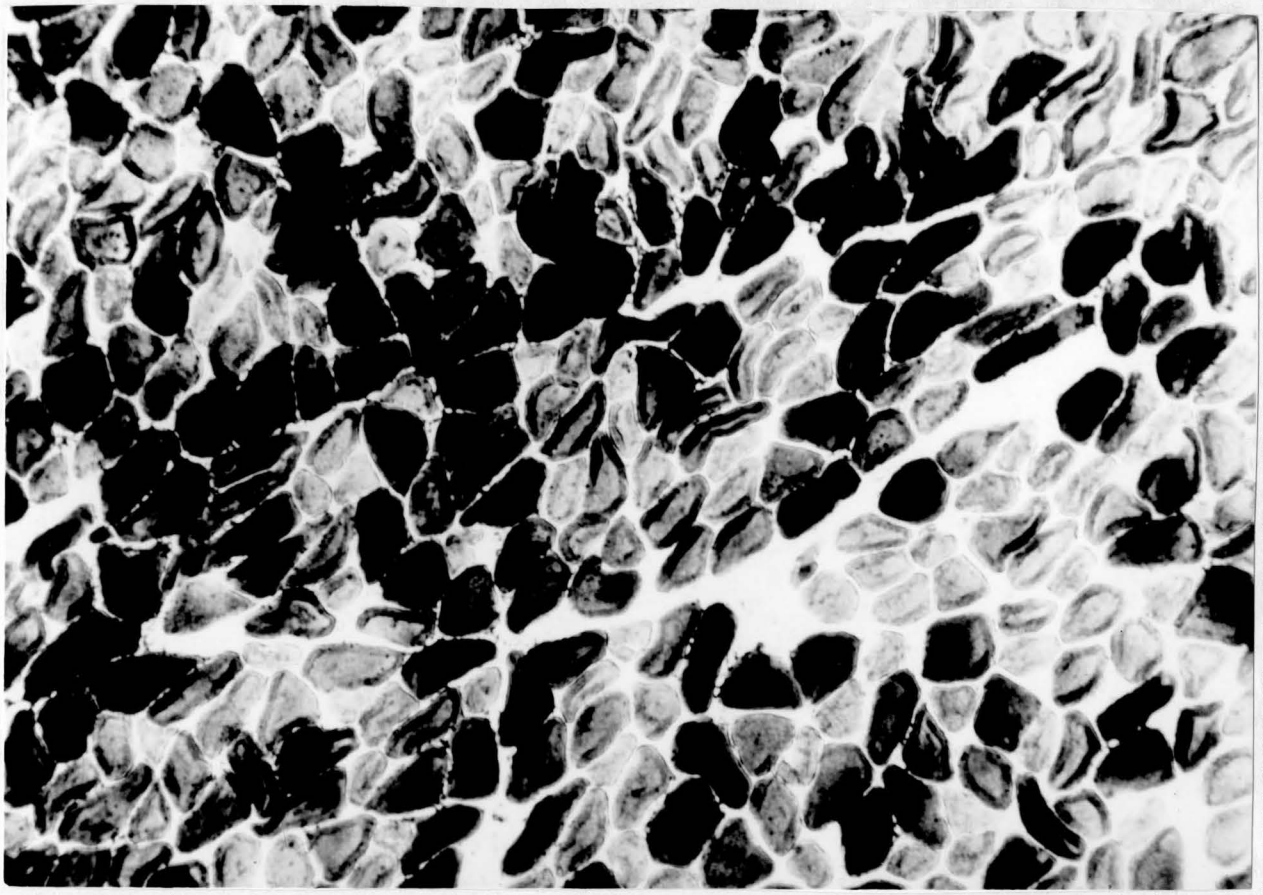

Fig. V. Incubation with Phosphorylase. Note the positive fibers. These may be either intermediate or white fibers.

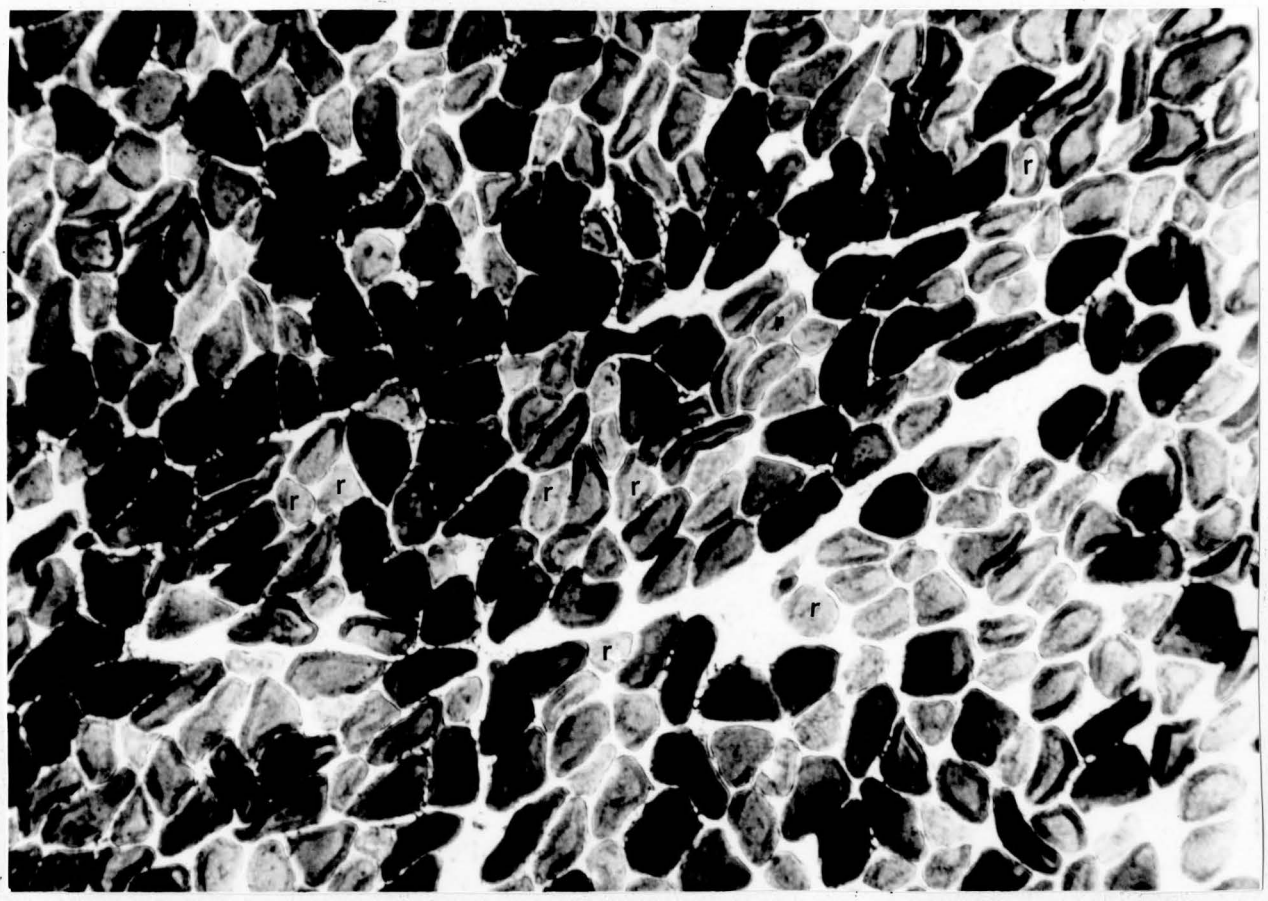

Fig. VI. Incubation with Phosphorylase. Note the negative fibers. These may be either intermediate or red fibers.

V. DISCUSSION

The presence of intermediate fibers was clearly shown in all the slides stained for myosin ATPase which has never been shown before in rat masseter muscle. Slides incubated for SDH also clearly showed the presence of intermediate fibers.

The confusion that has arisen in the past can be attributed to the fact that not all intermediate fibers are negative! That is they may be incorrectly called red or white fibers when the ATPase reaction is utilized.

The important thing is that all negative reactions for ATPase seem to consist of intermediate fibers. To identify the variable intermediate fibers, that is the ones that stain more positively for ATPase, consecutive sections must be used to compare each individual fiber in all three incubation media.

Phosphorylase is also variable for intermediate fibers. Therefore, fibers that have been labeled white in the past could have also been intermediate fibers. Fibers that react negatively to phosphorylase may also be intermediate fibers instead of red fibers (see Table I).

Therefore, rat masseter muscle is a heterogeneous muscle consisting of red, white and intermediate fibers. The muscle is capable of a combination of refined, quick and powerful movements, all of which are necessary to sustain life.

The importance of myosin ATPase in the metabolic pathways is well-known. However, when one tests for ATPase it is important to realize that it is required not only during muscle contraction, but also to carry out the necessary functions that allow the cell to remain living. That is one should

not forget that a muscle fiber is also a single, long, multinucleated cell. ATP enzymes are required both for cell function and muscle contraction.

It is interesting to hypothesize that the contraction times of intermediate muscle motor units is much slower. For example, in the rat soleus muscle the contraction time of 27 of 30 units were slow with contraction times of 38 msec. while the three remaining muscle fiber motor units were 18 msec. If the 27 of 30 units were intermediate fibers (this has been shown histochemically by Edgerton and Simpson (1969) and the remaining three units were red muscle fiber motor units, then the physiological data agrees with the histochemical data.

Many intermediate fibers are low in ATPase. Therefore, this might definitely explain their slower contraction and relaxation times. That is they do not have enough ATPase present to allow for a more rapid contraction. Temporomandibular joint dysfunctions may be attributed to the lack of sufficient fiber types to prevent the muscles of mastication, especially the lateral pterygoid from fatiguing and as a result, going into spasm. If the lateral pterygoid contains primarily intermediate fibers, their lack of ATPase and slower contraction times could lead to muscle imbalance and spasticity. An overabundance of a certain fiber type may also place the masticatory muscles and the buccinator in imbalance with the tongue or vice versa causing a muscle pressure imbalance and a resulting malocclusion, error in growth and development, or orthodontic relapse.

For example, if the masseter and internal pterygoid were functionally too strong, they could redirect the growth of the mandible and cause a horizontal growth pattern of the mandible. Also, the opposite might occur if these muscles were too weak and a vertical growth pattern with a skeletal open bite tendency might be the result.

If the obicularis oris were too strong, bony resorption of the anterior portion of the maxilla may be excessive and the resulting configuration of bone may be deficient for the anterior teeth causing early maxillary anterior tooth loss and a prognathic type of profile due to the predominance of the mandible. The evidence for this is already obvious in cleft palate patients who have an upper lip which is deficient or scarred. The vast majority of these patients also have a deficient maxilla. The upper lip also controls the position of the lower incisors. In cleft palate and prognathic mandible patients the lower incisors are tipped lingually.

In some cases the upper and lower arches are constricted before orthodontic treatment. After expanding this type of arch orthodontically, many of the patients relapse to their pretreatment constricted arch form.

The buccinator of these patients may have been too powerful because of an overabundance of red fiber types or some other unknown factor.

The variability of reaction of the intermediate fiber types leads one to believe the limitation of muscle fiber types into three catagories may be an oversimplification. Romanul (1964) described eight different subgroups utilizing eight different enzyme techniques. However, not all the muscles studied contained the eight different fiber types he described.

Therefore, he placed these subgroups into three larger groups to account for the differences.

As more knowledge about the histochemistry and physiology of muscle is obtained, it is hoped that a clinical application of these facts may be utilized. We must understand every aspect of muscle to assure out patients that our treatment will be stable and that they will be free of discomfort.

VI. SUMMARY AND CONCLUSIONS

This study was undertaken to specifically characterize the intermediate fiber of the rat masseter muscle. The intermediate fiber was identified in rat masseter muscle which to this date has not been reported in the literature. This was achieved by utilizing frozen sections and incubating them for ATPase, succinic dehydrogenase and phosphorylase.

The rat masseter muscle was characterized as a heterogeneous muscle consisting of intermediate, red and white muscle fiber types. This muscle should, therefore, be capable of all the movements necessary to sustain life.

A malformation of one or a group of the muscles of mastication might be a contributing factor to temporomandibular joint syndrome, errors in growth and development, malocclusion and orthodontic relapse.

BIBLIOGRAPHY

1. Baker, G. I.: Histochemical Characterization of the Muscles of Mastication: Thesis, University of Illinois, 1968.

2. Barka, T. and Anderson, P.: Histochemistry: theory, practice and bibliography, New York, Harper and Row, 1963.

3. Barnard, R. J., V. R. Edgerton, T. Furukawa, and J. B. Peter, Histochemical, biochemical and contractile properties of red, white and intermediate fibers. Am. J. Physiol. 220: 410-414, 1971.

4. Beatty, C. H., Basinger, G. M., Dully, C. C., and Bocek, R. M.: Comparison of red and white voluntary skeletal muscles of several species of primates. J. Histochem. Cytochem., 14: 590-600, 1966.

5. Chang, J. P. and Hori, S. H.: The section-freeze substitution technique: 1. Method. J. Histochem. Cytochem., 9: 292-300, 1961.

6. Close, R.: Properties of motor units in fast and slow skeletal muscles of the rat. J. Physiol. (London) 193: 45, 1967.

7. Close, R. I.: Dynamic Properties of Mammalian Skeletal Muscles. Physiol. Rev., 52: 129-197, 1972.

8. Cooper, S., and J. C. Eccles. The isometric responses of mammalian muscles. J. Physiol. (London) 69: 377-385, 1930.

9. Denny-Brown, D. E.: The Histological Features of Striped Muscle in Relation to its Functional Activity. Proc. Roy. Soc. (London), Ser. B 104: 371-411, 1929.

10. Diculesco, I., Onicesco, D. and Mischiu, L.: A histochemical analysis of dehydrogenase variety in the different types of muscular tissues. J. Histochem. Cytochem. 12: 145-152, 1964.

11. Dubowitz, V. and Pearse, A. G. E.: A comparative histochemical study of oxidative enzyme and phosphorylase activity in skeletal muscle. Histochemie. 2: 105-117, 1960.

12. Edgerton, V. R., and D. R. Simpson: The intermediate muscle fiber of rats and guinea pigs. J. Histochem. Cytochem. 17: 828-838, 1969.

13. Edstrom, L., and E. Kugelberg: Histochemical composition, distribution of fibers and fatiguability of single motor units. J. Neurol. Neurosurg. Psychiat. 31: 424-433, 1968.

14. Edstrom, L. and B. Nystrom: Histochemical types and sizes of fibers in

normal human muscles. Acta Neurol. Scand. 45: 257-269, 1969.

15. Engel, W. K.: The essentiality of histo- and cytochemical studies of skeletal muscle in the investigation of neuromuscular disease. Neurology 12: 778-784, 1962.

16. Engel, W. K., and G. Karpati. Impaired skeletal muscle maturation following neonatal neurectomy. Develop. Biol. 17: 713-723, 1968.

17. Eranko and Palkama, J. Histochem, Cytochem., 9: 585, 1961.

18. Eversole, I. R., and S. M. Standish. Histochemical demonstration of muscle fiber types. J. Histochem. Cytochem. 18: 591-593, 1970.

19. Feder, N. and Sidman, R. L.: Methods and principles of fixation by freeze-substitution. J. Biophys. Biochem. Cytol. 4: 592-600, 1958.

20. Fischer, H. Zur Physiologic der quergestreiften Muskeln der Saugertiere. Arch. Ges. Physiol. 125: 541-583, 1908.

21. Glick, D., and Fischer, E. E.: The histochemical localization of adenosine triphosphatase in plant and animal tissues. Science, 102: 429-430, 1945.

22. Gollnick, P. D. and Hearn, G. R.: Lactic dehydrogenase activities of heart and skeletal muscle of exercised rats. Amer. J. Physiol. 201: 694-696, 1961.

23. Gould, M. K. and Rawlinson, W. A.: Biochemical adaptation as a response to exercise. 1. Effects of swimming on the levels of lactic dehydrogenase, malic dehydrogenase and phosphorylase in muscles of 8, 11 and 15 week old rats. Biochem. J., 73: 41-43, 1959.

24. Greiff, D. and Kelly, R. T.: Cryotolerance of enzymes: 1. Freezing of lactic dehydrogenase. Cryobiology 2: 335-341, 1966.

25. Grutzner, P. Zur Anatomie und Physiologie der Quergestreiften Muskeln. Rec. Zool. Swisse 1: 665-684, 1884.

26. Hall-Craggs, E. C. B.: The contraction times and enzyme activity of two rabbit laryngeal muscles. J. Anat. 102: 241-255, 1968.

27. Hearn, G. R. and Wainio, W. W.: Aldolase activity of the heart and skeletal muscle of exercised rats. Amer. J. Physiol., 190: 206-208, 1957.

28. Herman, E. and Deane, H. W.: A comparison of the localization of alkaline glycerophosphatase, as demonstrated by the Gomori-Takamatsu method, in frozen and in paraffin sections. J. Cell. Comp. Physiol., 41: 201-223, 1953.

29. Hori, S. H.: Cytological phosphorylase locations in rat liver and muscle as shown by a lead precipitation method. Stain Techn., 39: 275-278, 1964.

30. Horwitz, C. A., Benitez, L. and Bray, M.: The effect of coenzyme Q on the histochemical succinic tetrazolium reductase reaction; a histochemical study. J. Histochem. Cytochem., 15: 216-224, 1967.

31. Howell, A. Brazier, Anatomy of the Wood Rat, 1926. The Williams and Wilkins Company. pgs. 27-29.

32. James, N. T.: Histochemical demonstration of myoglobin in skeletal muscle fibers and muscle spindles. Nature (London) 219: 1174, 1968.

33. Jinnai, D.: Functional differentiation of skeletal muscles. Acta Med. Okayama 14: 159, 1960.

34. Kendrick-Jones, J. and Perry, S. V.: Enzymatic adaptation to contractile activity in skeletal muscle. Nature, 208: 1068-1070, 1965.

35. Kleiner, I. S. and Orten, J. M.: Biochemistry. Chaps. 13 and 15, 6th ed., St. Louis, The C. V. Mosby Co., 1962.

36. Knoll, P.: Uber protoplasmaarme and protoplasmareiche muskulatur. Denkshr. Akad. Wiss. Wein. Mathnot. Kl., 58: 633-700, 1891.

37. Kronecker, H., and W. Stirling.: The genesis of tetanus. J. Physiol. (London) 1: 384-420, 1878.

38. Kuhne, W.: Ueber den Farbstoff der Muskeln. Arch. Lathol. Anat. 33: 79-94, 1865.

39. Lankester, E. R.: Ueber das varkommen von haemoglobin in den musklen der mollusken und die verbrietung desselben in den lebendigen organismen. Pflugers Arch. Physiol., 4: 315-324, 1871.

40. Maengwyn-Davis, G. D., Friedenwald, J. S. and White, R. T.: Histochemical studies of alkaline phosphatase in the tissues of the rat using frozen sections. II. Substrate specificity of enzymes hydrolyzing adenosine-triphosphate, muscle-and yeast-adenylic acids, and creatine-phosphate at high pH; the histochemical demonstration of myosin ATPase. J. Cell Comp. Physiol., 39: 395-447, 1952.

41. Malaty, H. A. and Bourne, G. H.: Histochemistry of succinic dehydrogenase Nature, 171: 295-297, 1953.

42. Melnick, P. J., Effect of freezing rates on the histochemical identification of enzyme activity. Fed. Proc., 24: 259-268, 1965.

43. Meryman, H. T.: General principles of freezing and freezing injury in cellular materials. Ann. N.Y. Acad. Sci., 85: 503-509, 1960.

44. Meyer, E. Ueber rothe und blasse quergestreifte Muskeln. Arch. Anat. Thysiol. 217-232, 1875.

45. Morell, S. A., Lipton, S. H. and Frieden, A.: Stability of disodium adenosine triphosphate. Science, 114: 333, 1951.

46. Nachlas, B. Histochem. Cytochem. 5: 420, 1957.

47. Novikoff, A. B., Shin, W. Y. and Drucker, J.: Cold acetone fixation for enzyme localization in frozen sections. J. Histochem. Cytochem., 8: 37-40, 1960.

48. Ogata, T.: A histochemical study of the red and white muscle fibers: 1. Activity of the succinoxydase system in muscle fibers. Acta. Med. Okayama, 12: 216-227, 1958.

49. Ogata, T. and Mori, M.: Histochemical study of oxidative enzymes in vertebrate muscles. J. Histochem. Cytochem., 12: 171-182, 1964.

50. Ohkawa, K.: Histochemical studies on the lipid requirement for the oxidative enzyme activities: 1. Studies on the effects of histological procedures for preparing tissue sections upon the oxidative enzyme activities. Acta. Tuberc. Jap., 15: 1-21, 1965.

51. Padykula, H. A. and Herman, E.: The specificity of the histochemical method for adenosine triphosphatase. J. Histochem. Cytochem. 3: 170, 1955.

52. Paukul, E.: Die Zuckungsformen von Kaninchenmuskein verschiedener Farbe und Structur. Arch. Anat. Physiol. 100-120, 1904.

53. Ranvier, L. Proprietes et structures differentes des muscle rouges et des muscles blanc chez les lapins et chez les raies. Compt. Rend. 77: 1030-1043, 1973.

54. Romanul, F. C. A.: Enzymes in muscle. I. Histochemical studies of enzymes in individual muscle fibers. Arch. Neurol. 11: 355-369, 1964.

55. Stein, J. M., and H. A. Padykula: Histochemical classification of individual skeletal muscle fibers of the rat. Am. J. Anat. 110: 103-124, 1962.

56. Stubbs, S. and Blanchaer, M. C.: Glycogen phosphorylase and glycogen synthetase activity in red and white skeletal muscle of the guinea pig. Canad. J. Biochem. 43: 463-468, 1965.

57. Takeuchi, T. and Kuriaki, H.: Histochemical detection of phosphorylase in animal tissues. J. Histochem. Cytochem. 3: 153-150, 1955.

58. Wachstein, M. and Meisel, E.: The distribution of histochemically demonstratable succinic dehydrogenase and of mitrochondria in tongue and skeletal muscle. J. Biophys. Biochem. Cytol., 1: 482-488, 1955.

59. Wade, H. E. and Morgan, D. M.: The analysis of adenosine triphosphate and adenosine diphosphate preparations by paper ionophoresis. Biochem. J., 56: 41-43, 1954.

60. Yellin, H.: Neural regulation of enzymes in muscle fibers of red and white muscle.. Exp. Neurol. 19: 92, 1967.

APPROVAL SHEET

The thesis submitted by Dr. Robert G. Thomas has been read and approved by the following Committee:

Dr. Patrick D. Toto
Chairman, Department of Oral Pathology, Loyola
Director of Graduate Studies

Dr. William F. Malone, Chairman
Fixed Prosthodontics
Assistant Dean, Loyola

The final copies have been examined by the director of the thesis and the signature which appears below verifies the fact that any necessary changes have been incorporated and that the thesis is now given final approval by the Committee with reference to content and form.

The thesis is therefore accepted in partial fulfillment of the requirements for the degree of Master of Science.

May 15 1975
Date

Patrick D. Toto
Director's Signature